REMARKS

Entry of the foregoing and reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested in light of the remarks which follow.

Claims 7, 13, 14, 17, 20 and 21 are amended herein, to capitalize "Tissue Factor", as requested by the Examiner. Thus, no new matter is presented by way of the present Amendment.

Claim Objections

Claims 7-17, 20, 21, 23-28, and 30-35 are objected to because the recitation of "tissue factor" purportedly refers to a particular protein thus should be capitalized. Claims 7, 13, 14, 17, 20 and 21 are amended herein to capitalize "Tissue Factor". Thus, Applications request that these objections be withdrawn.

Rejection under 35 U.S.C. § 112, First Paragraph

Claims 7-17, 20, 21, 23-28, and 30-35 stand newly rejected under 35 U.S.C. § 112, first paragraph. The Office Action states that the specification is enabling for activating blood vessel formation or enhancing wound healing in a subject in need comprising administering a nucleic acid expressing the Tissue Factor (TF) locally, wherein the nucleic acid is a plasmid vector comprising a constitutive promoter. However, the specification purportedly does not reasonably provide enablement for activating blood vessel formation

or enhancing wound healing in a subject in need, comprising inducing local expression of a TF by any means or locally administering any type of nucleic acid comprising an inducible promoter operably linked to a TF. Applicants respectfully traverse this rejection.

As, the Office is aware, "[a] patent need not teach, and preferably omits, what is well known in the art." *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986). The law does not require a specification to be a blueprint in order to satisfy the requirement for enablement under 35 U.S.C. § 112, first paragraph. Thus, not every last detail is to be described, else patent specifications would turn into production specifications, which they were never intended to be. *Staehelin v. Secher*, 24 U.S.P.Q.2d 1513, 1516 (Bd. Pat. App. & Int. 1992).

The Office Actions states that the specification relies on the state of the art as support for viral vectors, and that there are a variety of viral vectors in use (including retroviruses, adenoviruses, adenoassociated viruses, herpesviruses and poxviruses). It is further stated in the Office Action that "each system has perceived advantages and disadvantages, which influence their selection for current or projected clinical applications" (*see* pages 4-5 of Office Action). Applicants submit that these listed vectors were and still are the most relevant vector systems used in gene therapy. While each vector system may have specific advantages and disadvantages which may make one more appropriate than another for use in a specific application, all of them were and are successfully used in clinical gene therapy trials up to 2002 (see, for example, *The Journal of Gene Medicine Interactive Database*; www.wiley.co.uk/genetherapy/clinical). Their common use

emphasizes that each system can be used and no system is generally unsuitable or bears intolerable intrinsic safety risks.

Thus, Applicants submit that the skilled artisan would be able, at the time of filing of the present patent application, to select a suitable viral vector system to obtain enhanced overexpression of a Tissue Factor gene upon gene transfer for the claimed uses, including induction of wound-healing and the modification and induction of new blood vessels.

Further, Applicants submit that gene therapy and treatment using gene therapy have been under investigation for more than two decades. In the last twenty years, the spectrum of applications and the knowledge about viral vectors has significantly broadened. The body of experience and knowledge concerning all aspects of gene modifying and use of transfection shuttles was well advanced, enabling the skilled artisan to chose an effective transfection system for a given application.

For example, Verma *et al.*, (1997), *Nature* (1997) 18:239-42, discusses the strengths and limitations of all delivery systems (*see* Table 2). Important vector characteristics considered in Verma and Somia include maximum insert size, route of delivery, duration of expression *in vivo*, immunological problems or safety concerns.

Applicants now turn specifically to expression of Tissue Factor, and show that the local and transient expression of Tissue Factor afer gene transfer by means of a viral vector in a human is well understood in the art, as well as how the characteristics of a Tissue Factor application fit into the mentioned characteristics of different viral vectors.

Maximum insert size

The Tissue Factor cDNA is small (885 nt coding sequence), (see Morrissey *et al.*, *Cell*, (1987) 50:129-35). Therefore, a typical insert would not exceed the capacity of any viral vector for the transfer of a Tissue Factor gene. Molecular cloning for purposes of obtaining a virus capable of expressing a TF gene is a standard application well known in the art.

Route of delivery

Both *ex vivo* and *in vivo* are well known. For example, a successful approach is the use of *ex vivo* transfection of keratinocytes or myoblasts, as accomplished successfully with human Growth Hormone, Factor IX and Apolipoprotein E (see Vogt *et al.*, Proc. Natl. Acad. Sci. USA (1994) 91:9307-11). For this approach, the integration of viruses like retroviruses is also adequate to achieve local and transient expression. In addition, direct local *in vivo* delivery of the DNA is suitable, as used, for example, for VEGF expression (*see* Muehlhauser *et al.*, Circ. Res. (1995) 77:1077-86, Magovern *et al.*, Ann. Thorac. Surg. (1996) 62:425:33). Thus, in contrast to many other gene therapy applications, a transgenic virus can be delivered both ways for the process claimed in the present application.

Duration of *in vivo* transgene expression

With regard to the methods of the present invention, Applicants submit that the application of Tissue Factor does not require extensive expression time. The described approach of gene transfer via plasmid DNA has short duration of transgene expression, but is still proven to be very effective. The fact that a short *in vivo* transgene expression is sufficient for the application of a Tissue Factor gene actually facilitates the use of viral vectors, as compared to their use in other applications where long-term treatment would require multiple administrations and/or the construction of new viral vectors.

Immune reaction / general safety

For a viral vector, the immune reaction of the host against intruding viral proteins or against the protein expressed from the transferred gene is important. Adenoviruses are especially thought to provoke a specific immune response. However, a reaction against the product of the transgene is extremely improbable with regard to the claimed methods, because the Tissue Factor gene used and described in the patent application corresponds directly to the endogenous human cDNA. The patient in need of the claimed methods does not have a Tissue Factor gene defect. Thus, the TF protein is well tolerated by the immune system.

Applicants also emphasize that researchers using similar approaches often note a lack of a detectable immune reaction. Bellon *et al.*, *Hum. Gene Ther.* (1997) 8:15-25 discloses a phase I trial testing the expression of CFTR in cystic fibrosis patients. In this

topical application, "no significant deviations in immunological and inflammatory parameters were observed in serum and in bronchoalveolar lavage (BAL). Importantly, for all patients, the serum anti-adenovirus antibody levels did not change significantly from baseline and no antibodies against adenovirus were found in BAL." This affirms the results of an even earlier small study (1993) using CTRF, in which Zabner *et al.*, *Cell* (1993) 75:207-16 found no evidence of viral replication or virus-associated adverse effects.

With regard to wound healing, inflammation caused by viral vectors can be regarded as clinically non relevant, because an early inflammation reaction is regarded as an integral part of the wound healing process and therefore a relatively safe use was predictable. Sylvester *et al.*, *Wound Repair Regen.* (2000) 16:1993-8 state that "despite an increased acute inflammatory response after adenovirus injection, no difference in the healing capabilities of wounded skin was observed, suggesting that adenovirus-mediated gene transfer for growth factor-mediated acceleration of wound healing may be feasible". A similar opinion is expressed by Crombleholme, *Wound Repair Regen.* (2000) 8:460-72, stating "Secondly, as wound healing is fundamentally an inflammatory response, the inflammation elicited by adenovirus may not be detrimental as long as the transgene is a growth factor with significant vulnerary effects such as PDGF-B".

Moreover, many clinical trials underline the broad applicability of viruses reporting a good overall tolerability of gene therapy with various viral vectors in different therapeutic fields. The most well known gene therapy indication in which positive results are reported is cancer, i.e., prostate cancer (*see* Miles *et al.*, 2001, *Human Gene Ther.* (2001) 12:1955-

67); or lung cancer (*see* Tan *et al.*, *Anticancer Res.* (1996) 16:1993-8. Examples for other systemic approaches include treatment haemophilia (*see* Kay *et al.*, (*Nat. Geret.* (2000) 24:257-61). Favorable results were also obtained in the aforementioned topical application cystic fibrosis (*see* Zabner *et al.*, *Cell* (1993) 75:207-16. It is important to note that although many of the clinical studies were conducted recently, they are methodically based on techniques developed much earlier. Hence, they prove that experts in the field were able to use viral vectors for even more challenging applications regardless of their potential for immune responses.

Although the inflammatory response to viral vectors is of minor clinical concern for the claimed application, public awareness was drawn to this topic after the death of a patient in a gene therapy study was connected to the inflammatory response against adenovirus. Therefore, avoidance of viral vectors (and of the immune response against them) is an advantage more related to public attitudes rather than technical feasibility, safety and efficacy. An immune response is not a technical hurdle for the skilled artisan and would not prevent them from practicing the present invention.

Integration into host genome

Another point to consider is the usability of vectors which integrate into the host genome. Retroviruses, for example, integrate randomly, so especially for retroviruses there is a certain risk that by integrating somewhere a) a necessary gene is disrupted or b) a normally downregulated gene comes under the reign of the strong and/or constitutive

promoter of the construct (CMV in the given example). It is still not possible to estimate the probability of such an event, but it is expected to be "quite low" (*Verma and Somia*, 1997). Despite this concern, many clinical trials over a period of more than 10 years report no serious adverse effects caused by gene integration for example, Redfield *et al.*, N. Eng. Journal of Medicine (1991) 324: 1677-84 and "none of the theoretic safety hazards due to retroviral gene transduction was observed" (Merrouche *et al.*, *Journal Clin. Oncol.* (1995) 13:410-8.

Thus, Applicants submit there is sufficient information for the skilled artisan to perform transient and local expression of Tissue Factor by any gene transfer system, viruses as well as plasmids, for the use of viral systems is facilitated for the described application by several aspects:

- Small insert size
- Only limited expression time is needed
- Only local expression is needed
- *Ex vivo* as well as *in vivo* transfection is applicable
- Integrating vectors are usable especially for *ex vivo* transfection
- Overexpression of an endogenous protein prevents an immune reaction against the gene product
- Inflammatory responses are clinically acceptable, in particular if only local application is envisaged.

Claims 20, 21, 23-28, and 33-35 stand rejected for the recitation of a method comprising inducing local expression of a tissue factor nucleic acid in said subject. The Office Action states that given the broadest reasonable interpretation, the recited nucleic acid encompasses both endogenous and exogenous nucleic acids encoding TF, and claims encompass any means of induction of the TF expression.

The Office Action further states that although the specification contemplates using inducible or tissue specific promoters, the state of the art was not well developed at the time of the instant effective filing date, and cites to Miller *et al.* (*Hum Gene Ther*, 8: 803-15 (1997)). The Examiner asserts that it would require undue experimentation for the skilled artisan to practice the instant invention. Applicants respectfully traverse.

As stated in *Ex parte Forman* (230 U.S.P.Q. 546 (1986) the factors to consider in evaluating enablement and the need (or absence of need) for "undue experimentation" are the following: quantity of experimentation necessary, amount of direction or guidance presented, presence or absence of working examples, nature of the invention, state of the prior art, relative skill of those in that art, predictability or unpredictability of the art, and breadth of the claims. Applicants submit that the claimed invention is enabled.

The induction of local TF expression can be achieved by different means. Applicants will discuss the induction of the expression of a Tissue Factor gene controlled by tissue specific or inducible promoters and the elevation of expression of the endogenous Tissue Factor gene.

Concerning tissue specific approaches, many examples for such regulatory elements have been described before filing of the tissue factor application. By way of example, Applicants provide One example is the use of the alpha myosin heavy chain promoter, first described by Miller *et al.* in 1996. In 1997, Franz *et al.* used an adenoviral transfection system and observed no deregulation effect on the cardiac specificity of the promoter. Another example is the application of the prostate-specific antigen promoter (PSA-promoter). The promoter region was first described for its cell specificity in 1995 (Pang *et al.*).

Inducible promoters are also well known. One of the oldest inducible as well as repressible systems is the tetracycline dependent expression system (Gossen and Bujard, 1992). In fact, currently, the vectors can be purchased from BD Bioscience (www.bdbiosciences.com). Another example is the heat-inducible response elements from heat shock genes, first described by Derano *et al.* in 1986, which also proved to be effective in adenovirally transfected tumors. Further examples for inducible promoters include the ecdysone-inducible system (No *et al.*, 1996) and the radiation sensitive response elements (Boothman *et al.*, 1994). Both are described to perform well in viral gene transfer systems (Johns *et al.*, 1999).

An alternative to gene therapy for the activation of Tissue Factor expression would be the induction of the endogenous expression of Tissue Factor. Tissue Factor expression can be induced by a variety of agents including inflammatory cytokines (interleukin-1, tumor necrosis factor), mitogens (platelet-derived growth factor, epidermal growth factor,

transforming growth factor β-1, vascular endothelial growth factor, angiotensin II, thrombin), hormones, endotoxins, virus infections, immune complexes, modified lipoproteins, hypoxia and occupancy of cell adhesion molecules (Carson *et al.*, 1993; Lockwood *et al.*, 1993). Induction of endogenous expression of Tissue Factor therefore would be another way of achieving the sought after effect, as the latter is primarily dependent on the extent of expression.

Thus, Applicants submit that in light of the specification and what is known inthe art, the claims are enabled. Applicants request that the rejections under 35 U.S.C. § 112, be withdrawn.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

In the event any further fees are due to maintain pendency of this application, the Examiner is authorized to charge such fees to Deposit Account No. 02-4800.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

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By: _____
Deborah H. Yellin
Registration No. 45,904

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620